Using volatile organic compounds to monitor shelf-life in rocket salad

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Abstract

Rocket salad (*Diplotaxis tenuifolia* or *Eruca sativa*) is a perishable product of increasing interest due to its high content of nutritionally relevant compounds including glucosinolates and vitamin C. There is an increasing consumption of ready-to-eat salads which are sold to the consumer in bags, often packed under modified atmosphere. Shelf-life and sell-by dates are commonly applied to these products and are usually dictated by the appearance of the product rather than its nutritional value. During shelf-life, post-harvest deterioration leads to a loss of nutritionally relevant compounds such as vitamin C. This is accelerated by suboptimal conditions during storage and transport such as breaches of the cold-chain. Volatile organic compounds (VOCs) are easy and quick to sample use of thermal desorption gas chromatography time of flight mass spectroscopy (TD-GC-TOF-MS) enables remote sampling and a very sensitive analysis of VOC profiles. We have used TD-GC-TOF-MS to sample VOCs from rocket salad bags sourced from a local supermarket to assess changes during the shelf-life of the product. Using statistical analyses that treat the whole VOC profile as a single variable we show that it is possible to differentiate between day of purchase, use by date and time points beyond sale. We conclude that this methodology is therefore of use for assessing rocket salad quality through the supply chain.

Keywords: rocket salad, volatile organic compounds, shelf-life, gas chromatography, mass spectrometry, thermal desorption.

INTRODUCTION

Rocket salad is a widely consumed salad that provides a distinctive peppery flavor and is highly valued both for its taste and for its high content of nutritionally relevant compounds (Pasini et al., 2011). It is frequently sold alone or in mixed bags of ready-to eat salads which are widely consumed across Europe and world-wide. Nutritionally relevant compounds include ascorbic acid, carotenoids, phenolic compounds and glucosinolates (Bell and Wagstaff, 2014; Bell et al., 2015; Martínez-Sánchez et al., 2006). The latter are important compounds also for flavour development

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as they are broken down to isothiocyanates by the enzyme myrosinase when the leaves are subjected to mechanical damage such as chopping or chewing (Bennett et al., 2002, 2006). Both glucosinolates and isothiocyanates are reported to have nutritionally beneficial effects in protection against cancers and heart disease (Traka and Mithen, 2009). Other volatile organic compounds also contribute to the distinctive flavor of rocket salad and include nitrogen and sulphur containing compounds, alcohols, esters, and carbonyl compounds (Blažević and Mastelić, 2008; Miyazawa et al., 2002).

VOCs are easy to collect and measure, especially using thermal desorption which enables remote sampling and storage of the VOCs prior to sampling and is therefore well-suited to commercial quality-assessments (Spadafora et al., 2015). Using this approach 43 different VOCs were identified from stored rocket salad the major classes comprising aldehydes, sulphur compounds, ketones furanes and esters with lower numbers of alcohols, isothiocyanates, nitrogenous compounds, alkanes, alkenes and terpenes (Spadafora et al., 2016). Spadafora et al. (2016) were able to discriminate VOC profiles based on temperature of storage (between 0-10 °C) as well as time of storage over a 14 day period, and changes in VOCs were correlated to changes in nutritionally relevant compounds such as vitamin C and isothiocyanates.

However previous studies of VOCs in rocket salad have used material either grown directly in controlled growth conditions (e.g. Bell et al., 2014) or directly from a commercial source prior to packaging in commercial but known conditions (e.g. Spadafora et al., 2016). For a VOC-based analysis system to be applicable to the industry it must be robust enough to be applicable to any commercial sample without prior knowledge of its growth, processing or packaging conditions. We therefore used a combination of visual inspection and VOC analysis to assess quality in commercially sourced bagged rocket salad and demonstrate that the VOC system is indeed robust enough to discriminate between day of sale, use-by date and time points beyond marketability of the product.

MATERIALS AND METHODS

Plant material and storage conditions

Twelve bags of prepared rocket salad (*Diplotaxis tenuifolia*) were obtained from a local supermarket at Cardiff and stored for the period up to their “use by” date as stated on the bag at a storage temperature of 7 °C, and then for a further 9 days.

Visual assessment of quality

The rocket salad was assessed visually based on a rating scale for rocket salad (*Diplotaxis tenuifolia*) for limit of marketability and limit of edibility (Amodio et al., 2015) by three independent laboratory-trained assessors. The scale ranges from 1 to 5 where 1 = evident defects, 2 = poor with major defects, 3 = fair with acceptable defects, 4 = intense aroma when crushed with slight defects, 5 = fresh unblemished appearance with intense aroma when crushed. A score of 2 is considered to be the limit of edibility and a score of 3 for the limit of marketability.

Collection and analysis of VOCs

At each time point 100 g of rocket leaves were removed from the commercial bag and placed in a multi-purpose roasting bag (25 cm x 38 cm, TJM Ltd). The bag was sealed using an elastic band and the sealed bag was then lightly crushed to release the VOCs. The head-space was then equilibrated by storing the bag at 20 °C for 1 h. Samples of 1000 mL of headspace were collected onto SafeLok thermal desorption tubes (Tenax TA and Sulficarb, Markes International Ltd.) using an EasyVOC manual pump (Markes International Ltd.). Control samples consisted of an equal volume of air collected from empty bags at the same time. Triplicate samples were taken for each time-point using individual independent bags of the rocket salad. Tubes were desorbed on a TD100 thermal desorption system (Markes International Ltd.). The following settings were applied for the tube desorption: 5 min at 100 C then 5 min at 280 °C, a trap flow of 40 ml/min and trap desorption and transfer: 20°C/s to 300 °C, split flow of 5 ml/min into GC (7890A; Agilent Technologies, Inc.). VOCs were then separated over 60 m, 0.32 mm I.D., 0.5 µm Rx5ms (Restek) using the following temperature programme: initial temperature 40 °C for 2 min, 5 °C/min to 240 °C, final hold 5 min. Retention standards were prepared by loading 1 µL C8-C20 alkane standard onto a TD tube.

Mass spectra were recorded from m/z 30 – 350 on a time-of-flight mass spectrometer (BenchTOF, Markes International Ltd). The data from the GC-MS measurements were deconvoluted, and compounds identified and integrated with AMDIS (NIST11) using a custom retention-indexed mass spectral library. Compounds, which were not detected in all replicates, were excluded from further analysis as were compounds that were abundant in controls. Only compounds which scored > 80 % in both forward and backward fit were included within a custom mass spectral library. Putative identifications of compounds were based on matches of their mass spectra (> 80 %) and a retention index (RI +/- 15). Areas of the remaining compounds were normalized within samples and were standardised for each compound.

Statistical analysis

Statistical analyses of the VOC data were performed using R software version 3.1.3 (R core development team 2015) following normalisation of areas and square root transformation in order to reduce weight of large components. Permutational multivariate analysis of variance (PerMANOVA) and Canonical Analysis of Principal coordinates (CAP) analysis (Anderson and Willis, 2003) were both carried out using the 'vegan' package (Oksanen, et al., 2013) and the 'BiodiversityR' package (Kindt and Coe, 2005) within R. Ordination plots were generated for storage day and a confidence interval (95%) was fitted to the data. Analysis of abundance of individual VOCs is based on an ANOVA followed by a Tukey's test.

RESULTS AND DISCUSSION

Sensorial quality of commercially obtained rocket salad samples

The bagged rocket salad was assessed for sensorial quality markers on the day of purchase and at intervals through its storage at 7 °C (Fig. 1). On day of purchase the leaves were of a deep green with no visible defects and with the intense characteristic aroma of rocket salad when crushed (score 5). In line with the use-by date, as marked on the bag, the salad was still of acceptable visual quality with intense aroma when the leaves were crushed (score 4 = slight defects) after 3 days storage. Very little change was visible between 3 and 10 days, however, after 10 days storage at 7 °C, the score had dropped to below marketability with a score of 2 (poor with major defects) and by day 12 the leaves had a score of 1 (inedible, very poor with evident defects). At the 12-day time point, there was also the appearance of a strong off-odour. Leaves from 4 bags were examined at each time point: no appreciable difference was evident amongst different bags at the same time point. The drop in quality is in line with previous reports (Amodio et al., 2015; Spadafora et al., 2016) which showed that at 5 °C the limit of marketability was reached after 5.8 days and if stored at 15 °C after 3.7 days. These results therefore indicate that prior to purchase, the bagged rocket salad had probably not been subjected to severe anomalous stresses such as breaks in the cold chain, which are known to reduce shelf life (Amodio et al., 2015).

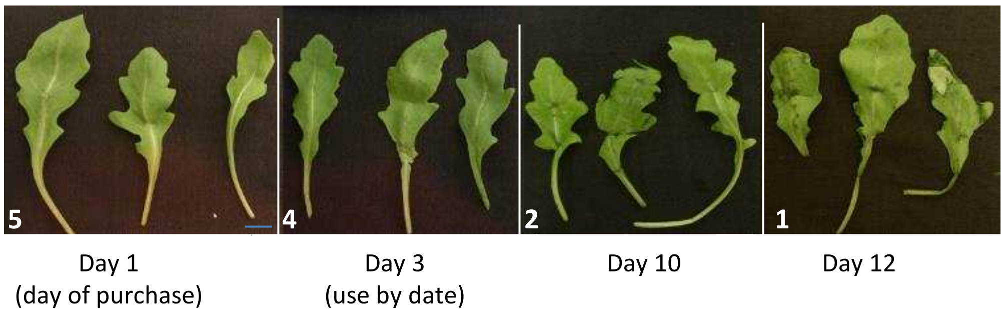

Figure 1. Changes in rocket leaf appearance of bagged samples obtained from a high quality supermarket local to Cardiff University and monitored through shelf-life stored at 7°C. Quality score is noted on the bottom left hand corner of the image for each day.

VOCs identified from the rocket salad samples

A total of 41 VOCs were detected in the salad bags (Table1). A similar distribution of compound classes was obtained compared to a previous report using this VOC collection and analysis method (Spadafora et al., 2016) in which 43 VOCs were identified. However, the identity of the compounds in each class was substantially different: 34 % of the compounds identified here were not found in the previous report. Major differences were a lack of terpenes in this study and a greater number of isothiocyanates (five compared to only three in Spadafora et al., 2016). This suggests that differences in the cultivar of salad used, the environmental conditions pre-harvest and/or the commercial treatment applied to these samples during processing and transport/storage elicited differences in the volatile bouquet. This is important in considering the use of the VOC analysis system for assessing quality in commercially obtained samples with no prior knowledge of their packaging or conditions of transport and storage.

Analysis of VOCs for discriminatory power between days of storage

Statistical analyses were applied to analyse the differences in VOC profiles between time points. PerMANOVA analysis was selected as this treats the whole profile as a single variable and hence considers the possibility that changes in each VOC may not be independent of other VOCs dues to the complexity of plant secondary metabolism.

VOC profiles were discriminated by day of storage. Overall the PerMANOVA analysis accounted for 83.4 % of variability of the entire sample set. Linear discrimination plots were constructed using CAP analysis and showed clear discrimination between day 1 and day 3 of storage, which correspond to day of purchase and sell-by date ($P < 0.0005$)

and both of these were discriminated from days 10 and 12. However, day 10 and day 12 were not discriminated from each other (91.6% correct classification at $P < 0.005$; Fig. 2). At the later time points there was a detectable development of off-odours and the leaves had clearly deteriorated beyond marketability and edibility.

Spadafora et al. (2016) identified a group of seven aldehyde VOCs that correlated with a fall in vitamin C. All seven of these aldehydes are also found amongst the VOCs identified from the bagged salad here. A comparison of their relative abundance across the time course shows that the relative abundance of all seven VOCs is also negatively correlated with time in the bagged salad (Fig. 3) with all the compounds being detected in days 1 and 3 but not thereafter.

Table 1 VOCs detected in rocket salad bags obtained from a local CU retailer

Chemical group	**VOC***
Aldehyde	4-Oxohex-2-enal
	2,4-Hexadienal
	2-Methyl-2-butenal
	(E/Z) -2-Hexenal,
	(E/Z)-2-Hexenal
	2-Pentenal
	(E/Z)-3-Hexenal
	(E/Z)-3-Hexenal
Alcohol	***3-methyl-1-Butanol***
	1-Penten-3-ol
	2-Penten-1-ol-
	3-Hexen-1-ol
Alkane	2,4-Dimethyl-undecane
	1-Chloro-decane
	Eicosane
	2,6,10,14-Tetramethyl-hexadecane
Alkene	***1-Nonene***
	3-Undecene
Ketone	1-Penten-3-one
	3-Pentanone
Ester	3-Hexen-1-ol acetate
	2-oxo-Hexanoic acid methyl ester
	n-Propyl acetate
	Oxalic acid diallyl ester
Isothiocyanate	4-Methylpentyl isothiocyanate
	1-Isothiocyanato-hexane
	n-Pentyl isothiocyanate
	1-Isothiocyanato-3-(methylthio)-propane
	Thiocyanic acid methyl ester
Oxime	5-Nonanone oxime
Amine	***Cyclobutylamine***
	Methenamine
Propane	***Cyclopropane ethylidene***-
Sulphur containing	Dimethyl sulfide
	Dimethyl sulfoxide

	Dimethyl trisulfide
	Dimethy disulfide
	Dimethyl disulfide
	Tetrahydro-thiophene
Furan	2-Ethyl-furan
	5-ethyl-2(5H)-Furanone

*VOCs in bold italics were not identified in Spadafora et al. (2016)

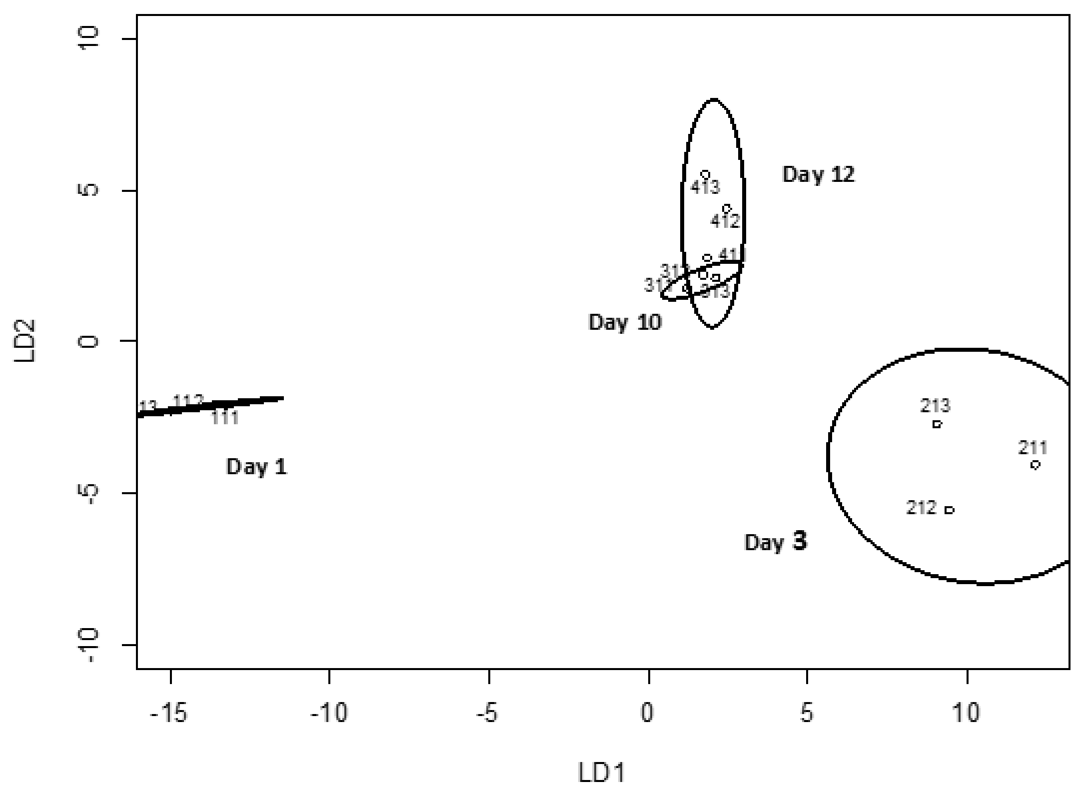

Figure 2. Canonical analysis of principal coordinates of TD-GC-TOF-MS detected compounds in bagged rocket samples during shelf-life assessment. A CAP model was produced for rocket leaf samples stored at 7°C for 1, 3, 10 and 12 days. Each ellipse represents the 95% confidence interval. The CAP model was based on the first two linear discriminants (LDs). LD1 was plotted against LD2 to produce LD loading plot, with a percentage of correct classification = 91.6% ($P < 0.005$; n=3).

Thus these aldehyde VOCs show a strong indication of being robust as markers of an acceptable overall quality. More extensive sampling and a more detailed time course will be required to validate their use as markers for nutritional quality.

Furthermore a rise in three sulphur containing compounds, dimethyl sulfoxide, dimethyl sulphide and dimethyl disulphide was associated by Spadafora et al. (2016) with a rise in off-odours and microbial growth. All three of these compounds were identified here amongst the bagged salad VOCs. Abundance of these VOCs also followed the same pattern as found previously, indicating that again they can be correlated with an increase in off-odours.

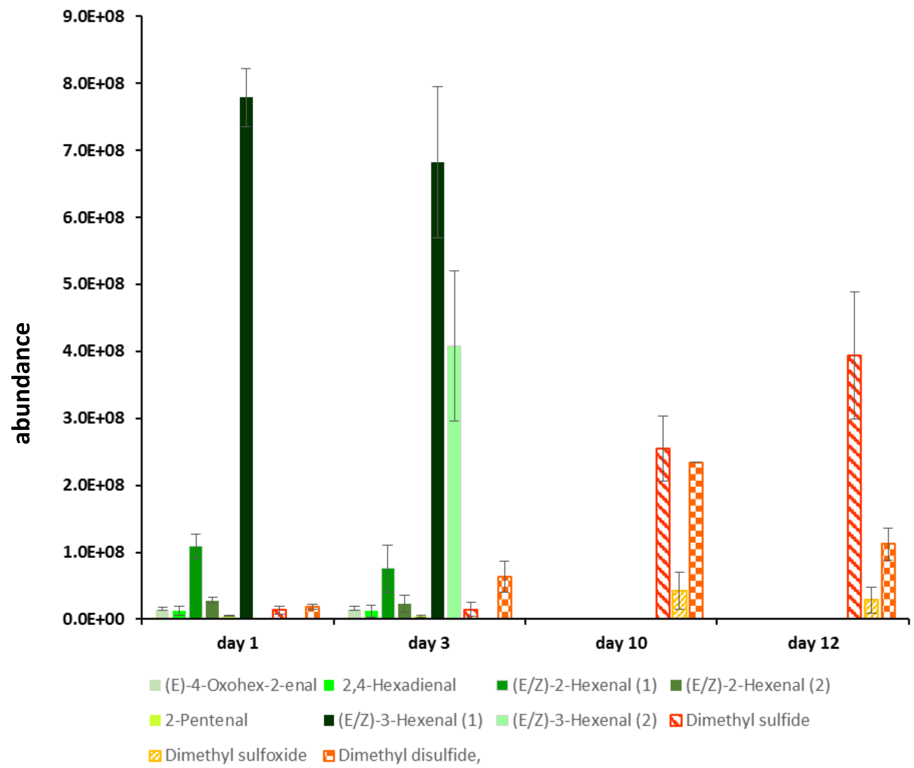

Figure 3. Abundance of seven aldehydes (solid shading) and three sulphur containing (patterned shading) VOCs over time in bagged rocket salad stored at 7°C. Mean abundance ± S.D., n=3.

CONCLUSIONS

We clearly demonstrate that the TD-GC-TOF-MS collection and analysis method for VOCs is robust enough to discriminate between rocket leaves stored at a commercially relevant temperature and which are visually both of acceptable marketability and edibility. Furthermore we show that despite significant differences in the VOC profile between studies, the analysis is sufficiently robust to still discriminate times of storage thus demonstrating that this approach has considerable potential as a quality assurance tool in the fresh cut industry.

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